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Stem cell therapy for Alport syndrome: the hope beyond the hype*

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Alport syndrome is a hereditary glomerulopathy leading to end-stage renal disease (ESRD), frequently during adolescence. It is caused by the absence or abnormal composition of the type IV collagen α3/4/5 network normally present in the glomerular basement membrane (GBM) [1]. In the September 2008 issue of the *Journal of the American Society of Nephrology*, Katayama and colleagues reported that bone marrow transplantation (BMT) following lethal irradiation with either wild-type or *Col4a3*−/− BM prolonged the lifespan of Alport mice with similar efficiencies. Sublethal irradiation alone also provided significant benefits [2].

These results challenge reports from Cook's and Kalluri's groups suggesting that wild-type BM-derived cells ameliorate disease in Alport mice because they can differentiate into podocytes and secrete the missing collagen α3/4/5(IV) chains [3,4], which would basically constitute a curative cell-based therapeutic approach for treating Alport glomerulopathy. Their interpretation that circulating BM-derived cells are recruited to damaged glomeruli where they can cross the GBM, become podocytes, secrete the missing collagen chains, repair the GBM defects and slow—if not reverse [4]—disease progression appeared (and still appears) incredibly fascinating. However, although these authors reported improvements in overall kidney histology compared with untreated or *Col4a3*−/− BM-treated Alport mice, the most meaningful endpoint, i.e. age at ESRD, was not tested in either study for reasons not explained [3,4]. This issue is critical, because a previous study of Alport mice that received BM-derived mesenchymal stem cells also improved renal histology, but there was no delay of death from renal failure [5].

One potentially important difference between the studies of Katayama *et al*. and of Prodromidi *et al*. as well as Sugimoto *et al*. is the genetic background of the Alport mice used. Progression of Alport's disease in mice is influenced by the genetic background [6]. A mixed genetic background carries the risk of spoiling the most meaningful endpoint, i.e. the age at ESRD (Figure 1). The increased lifespan in C57BL/6J Alport mice may be explained in part by an 'escape phenomenon' characterized by an alternative collagen switch (Figure 2), whereas the GBM of the C57BL/6J Alport mice used by Prodromidi *et al*. and Sugimoto *et al*. [3,4] may be stabilized by incorporation of α5/6(IV) chains,

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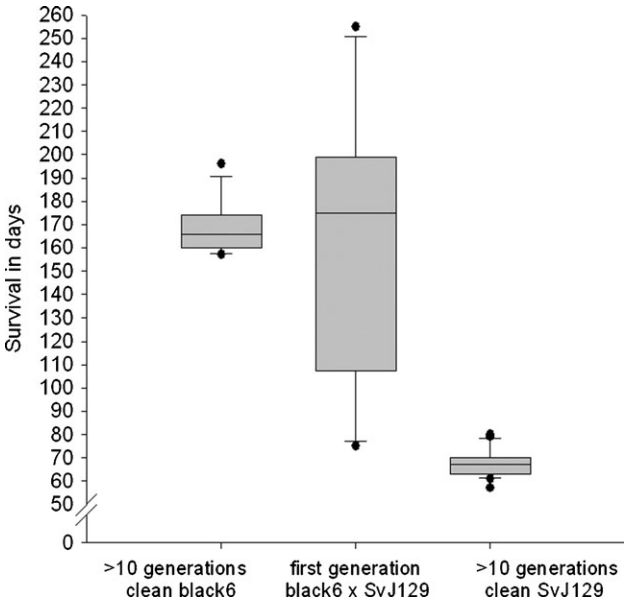

Fig. 1. Age at death from renal failure in *Col4a3*−/− mice. Lifespan depends on the genetic background. Furthermore, the standard deviation in lifespan strongly depends on a clean genetic background (see box plot in the middle, crossbreeding of C57BL/6J and 129 × 1/SvJ-backgrounds). The immense standard deviation in an unclean background might confound interpretation of experiments, including histological evaluations.

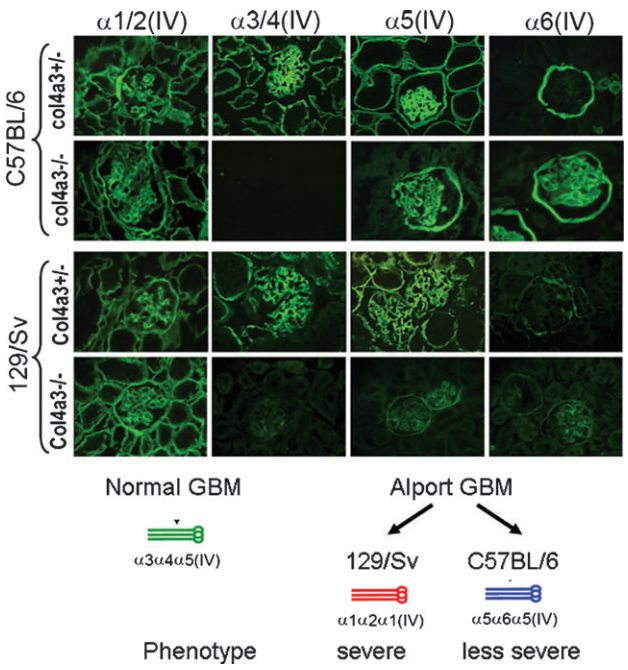

Fig. 2. Immunostaining of different α(IV) collagen chains in Alport mouse kidneys. The GBM deposition of the α3/4/5(IV) chains depends on the gene defect (heterozygous or homozygous state) as well as on the genetic background. Note the 'escape phenomenon' within the GBM of C57BL/6J *Col4a3*−/− mice: these mice incorporate significant amounts of α5/6(IV) chains into their GBM. This phenomenon might contribute to the improved lifespan of Alport mice on this background (Figure 1) as compared to the 129 × 1/SvJ Alport mice, which show much less α5/6(IV) incorporation.

possibly increasing the lifespans of the mice. The GBM of the 129 × 1/SvJ Alport mice used by Katayama *et al*. [2] exhibits far lower (though still detectable) incorporation of the α5/6(IV) chains, which correlates with their rapid progression to ESRD [7].

Cell-based therapies aim towards repairing the underlying defect: here, the defective assembly of α3/4/5(IV) collagen. Katayama *et al*. certainly shared this aim, but they found no deposition of α3/4/5(IV) collagen in their WT BM-transplanted Alport mice, despite the extended lifespan [2]. We disagree with the claim in LeBleu and Kalluri's recent editorial [8] that 'careful examination of immunohistochemistry and Western blot images in the study by Katayama *et al*. reveals a likely faint labelling for α3 chain'. Although both Prodromidi *et al*. and Sugimoto *et al*. concluded that α3(IV) was present in the GBM of their BM-transplanted Alport mice [3,4], neither presented definitive supporting evidence. Focal staining for α3(IV) [3,4] does not prove true GBM deposition; patients with X-linked Alport syndrome show intracellular α3(IV) mRNA and protein in podocytes that is unaccompanied by α4 or α5(IV) [9], and similar findings have been reported in Alport dogs [10]. Furthermore, widespread linear staining for α5(IV) in the GBM of Sugimoto *et al*.'s treated Alport mice, which they presented as evidence for α3/4/5(IV) deposition [4], might then be irrelevant due to the alternative switch [7] discussed above. Surprisingly, Sugimoto *et al*.'s untreated *Col4a3*−/− mice did not show GBM staining for α5(IV) [4]. This might be explained by an impure genetic background, with the untreated *Col4a3*−/− mice having some contribution from a 129 × 1/SvJ (129) or another background. This could also explain their worse renal function as compared to the BMT group, which shows the alternative switch.

A convincing demonstration of assembly of the correct GBM collagen IV network requires biochemical evidence of α3/4/5(IV) chain co-assembly, such as by assaying that chains are co-immunoprecipitated by monoclonal antibodies with well-defined α(IV) chain specificities. Even assuming that small amounts of α3/4/5(IV) were deposited in the *Col4a3*−/− mouse GBM after BMT [3,4], it is unlikely that such a focal restoration of the correct network would improve renal survival. Indeed, insufficient incorporation of α3/4/5(IV) into the GBM can lead to severe kidney involvement in *Col4a5*+/− female mice, in which ∼50% of podocytes are fully capable of producing the genuine α3/4/5(IV) collagen network [11]. Nevertheless, Sugimoto *et al*. did detect α3/4/5(IV) in whole kidney lysates. Western analyses show up to half as much α3/4/5 noncollagenous (NC1) domain monomers in the transplanted mutant kidneys as compared to the wild-type control kidneys. Given that the α3/4/5(IV) network is found throughout the cortex in proximal tubules and in collecting ducts, as well as in the GBM, widespread incorporation of collagen IV-secreting transplanted cells throughout the parenchyma would be needed to achieve such levels. Analysis of isolated glomeruli would be necessary to provide convincing evidence of incorporation of the α3/4/5(IV) network in GBM and this was not performed [4].

Katayama *et al*. show that irradiation alone provides benefits without BMT [2], implying that a significant

portion of the amelioration of disease reported by Cook's and Kalluri's groups [3,4] might have little to do with the transplanted cells. Some statements (one of them quoted above) in LeBleu and Kalluri's recent editorial [8] on Katayama's paper appear to be designed to weaken its impact. As the data of Katayama *et al*.'s study challenge the proposed concept/mechanism of BMT therapy for Alport syndrome, a careful analysis of their data and their approach is necessary to avoid further controversy with regard to the importance of these studies:

1. Katayama's study was performed in Col4a3−/− mice on the 129 × 1/SvJ background, which renders comparisons to data generated on the C57BL/6J background potentially problematic. The age at the time of BMT also differed among the studies. The different backgrounds and ages at BMT could be responsible, in part, for the different outcomes, given differences in timing and severity of GBM defects and their potential effects on the behaviour of the transplanted BM cells. Indeed, LeBleu and Kalluri suggest that Katayama's 129 × 1/SvJ Alport mice, transplanted at 3 weeks, would not have the GBM defects that are 'a likely prerequisite for successful cell-based therapy' [8]. Yet results in the two references cited [12,13] and others [6] indicate that it is highly unlikely that at 3 weeks of age the Alport GBM on the 129 background is normal. More importantly, there is no evidence to support the assumption that GBM damage is a pre-requisite for a stem cell repopulation.
2. Katayama *et al*. did not directly compare treatment with and without BMT at a given irradiation dose. However, the finding that Col4a3−/− mice transplanted with Col4a3−/− BM after irradiation with 8 Gy (stands for Gray, irradiation dosage) live longer than non-transplanted Col4a3−/− mice irradiated with 3, 6 or 7 Gy (stands for Gray, irradiation dosage) does support the author's interpretation of a dose-dependent effect of irradiation.
3. Katayama *et al*.'s analysis of α(IV) chain expression was limited to 7- and 11-week-old mice, only 4 or 8 weeks after BMT. The study lacks an additional later time point such as 15 weeks of age (12 weeks after BMT). This later time point would have allowed us to exclude the existence of incomplete BM reconstitution and later progenitor cell migration into the kidney, which might have been missed at the earlier time points.
4. Katayama *et al*. do not provide data on albuminuria, but the BUN and serum creatinine levels (being highly correlated with albuminuria in the Sugimoto study) convincingly demonstrate improvement of renal function.
5. Finally, Le Bleu and Kalluri raised doubts [8] about Katayama's finding that irradiated mice lived longer independent of a significant effect on interstitial fibrosis. However, previous studies in Col4a3−/− mice on the 129 background have already demonstrated that the extent of interstitial fibrosis does not correlate with survival time [5].

In summary, despite some limitations underscoring our lack of basic understanding of this emergent area of research, the study by Katayama clearly demonstrates that BMT as a collagen IV replacement therapy does not provide additional benefits beyond irradiation to prolong the survival of *Col4a3*−/− mice on the 129 × 1/SvJ genetic background. In agreement with a previous editorial [8], future studies in this field should use Alport mice with a clean genetic background. Most importantly, histological studies should be supported by showing the numbers of animals treated and survival curves, including standard deviations in both, with treated and untreated mice being littermates of each other.

Taking the above *conditio sine qua non* into account, *Col4a3−/− mice* have been particularly useful for testing potential therapies. As a potentially life-threatening procedure, BMT will need to be demonstrated to be superior to less dangerous drug-based therapies for Alport nephropathy, some of them being broadly used as off-label therapies throughout the world. European and American Alport registries currently investigate the long-term outcome and the safety of these medications. Unfortunately, scientific articles on the preliminary and controversial basic research in Alport-mice may be misinterpreted as 'a cure has been found' and editorial headlines that try to turn doubts about the concept into a proof of the desired truth are misleading and have had unfavourable secondary effects [3,4,8]. In fact, the lay press turned the hope of a BMT cure for Alport glomerulopathy into hype. This creates a difficult situation for clinicians taking care of Alport patients: hopes that clinicians cannot fulfil put an unfortunate burden on nephrologists dealing with parents who might be desperate because of their child's impaired renal function and who might be ready to take every chance to help their child. Such hopes might turn into unjustified risks!

Our current knowledge about Alport syndrome and its possible therapy by BMT is as yet incomplete and reveals many open questions that need to be addressed experimentally before clinical BMT studies should be considered in Alport patients. Due to the associated risks of the BMT procedure, it is currently only being performed in potentially lethal diseases. In fact, given the perspective of renal transplantation, Alport syndrome may not generally qualify for BMT in terms of improving patient survival. Thus, any experimental doubt that the effects of BMT are superior to those of other treatments (like drugs or irradiation) increase the need for open-minded discussions and more research before taking inappropriate risks that could eventually harm our young patients.

Conflict of interest statement. C.K. is the executive director of the Alport Syndrome Treatments and Outcomes Registry (ASTOR), USA. O.G. is the principal investigator of the European Alport therapy registry, supported by the Association pour l`Information et la Recherche sur les maladies rénale Génétiques France and the KfH-Foundation Preventive Medicine.

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